[Biochemistry]

LIPID ORDERING BY CHOLESTEROL IN GIANT UNILAMELLAR VESICLES

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Cholesterol plays a critical role in the plasma membranes of mammalian cells by adjusting the physical properties and enzymatic activities of the lipid bilayer. Investigations with nuclear magnetic resonance and other spectroscopic techniques have shown that cholesterol significantly affects the chain conformation in the lipid bilayer. However, such studies were performed exclusively upon multilamellar vesicles that consisted of many concentric bilayers. Inter-bilayer interactions make results from these multilamellar samples difficult to reconcile with theoretical studies usually performed on single bilayers.

To mimic the plasma membrane more closely, giant unilamellar vesicles (GUV's) were prepared from DPPC mixed with cholesterol at various concentrations and fluorescently labeled with DPPE–rhodamine. Polarization fluorescence images of GUV's at the equatorial plane were acquired by laser-scanning microscopy. The ordering degrees of the fluorescent probes were calculated from the intensity dependence on the excitation polarization.

The obtained images clearly show that cholesterol fluidizes the DPPC bilayer at 21 °C and that the ordering of DPPC lipids decreases with increasing cholesterol content. Although no macroscopic domains appear to have formed in cholesterol percentages of up to 50%, a rapid drop in the lipid ordering degree is seen when the cholesterol percentage is higher than 35%. This result provides experimental evidence for the existence of 1:2 stoichiometric complexes in DPPC/cholesterol bilayers.